

EFFLUENT SAMPLE SITES:

- A = Industry A
- B = Municipal WWTP A
- C = Industry B
- D = Industry C
- E = Municipal WWTP B

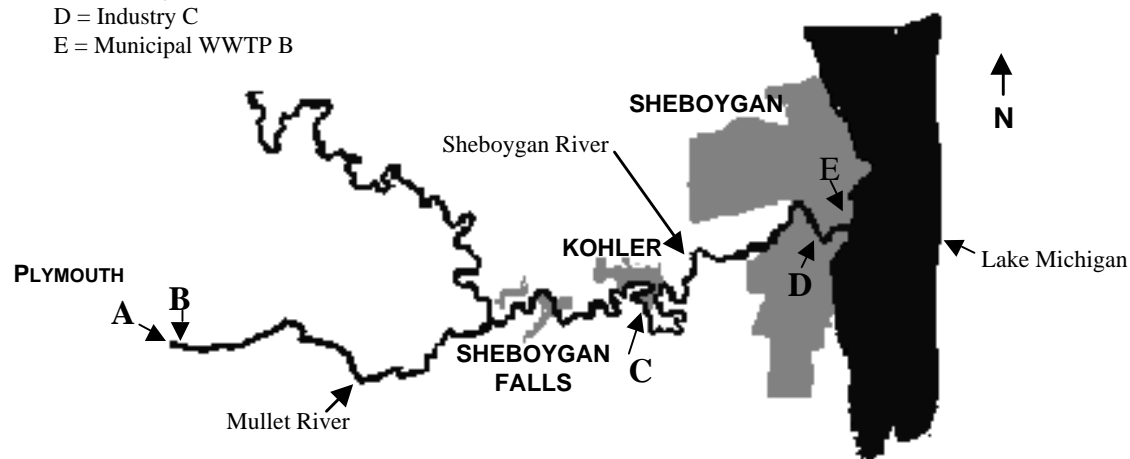


Figure 1. Map showing approximate locations of facilities where effluent samples were collected. Sediment was collected upstream and downstream of each point source discharge, except that no sediment was collected downstream of municipal WWTP B.

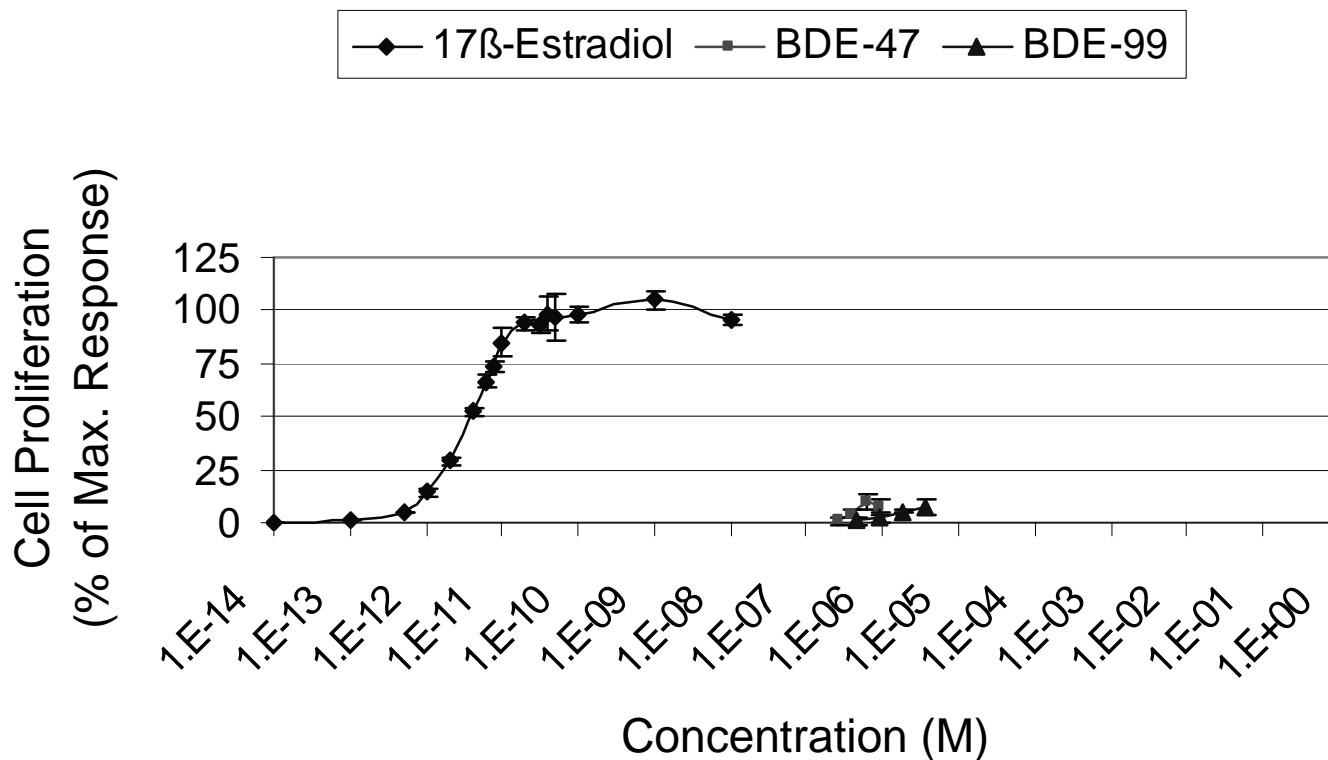


Figure 2. Proliferation of MCF-7 cells exposed to indicated concentrations of 17β-estradiol, BDE-47 and BDE-99. Maximum cell proliferation of 17β-estradiol was set as 100%. Cell number after five days treatment of MCF-7 cells with BDE-47 or BDE-99 at the indicated concentrations is expressed as percent of maximum response relative to the positive controls (17β-estradiol spikes) and negative controls (ethanol- only(?) blanks) as follows: % of maximum response =  $(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) / (\text{Abs}_{17\beta\text{-estradiol spike}} - \text{Abs}_{\text{blank}}) * 100$ , where Abs = absorbance. Each point is the mean  $\pm$  standard deviation ( $n=4$ ). The concentration of 17β-estradiol that caused 50% of the maximum proliferation response, or  $\text{EC}_{50}$ , was 3.7 pM (Softmax PRO v. 2.6 software).

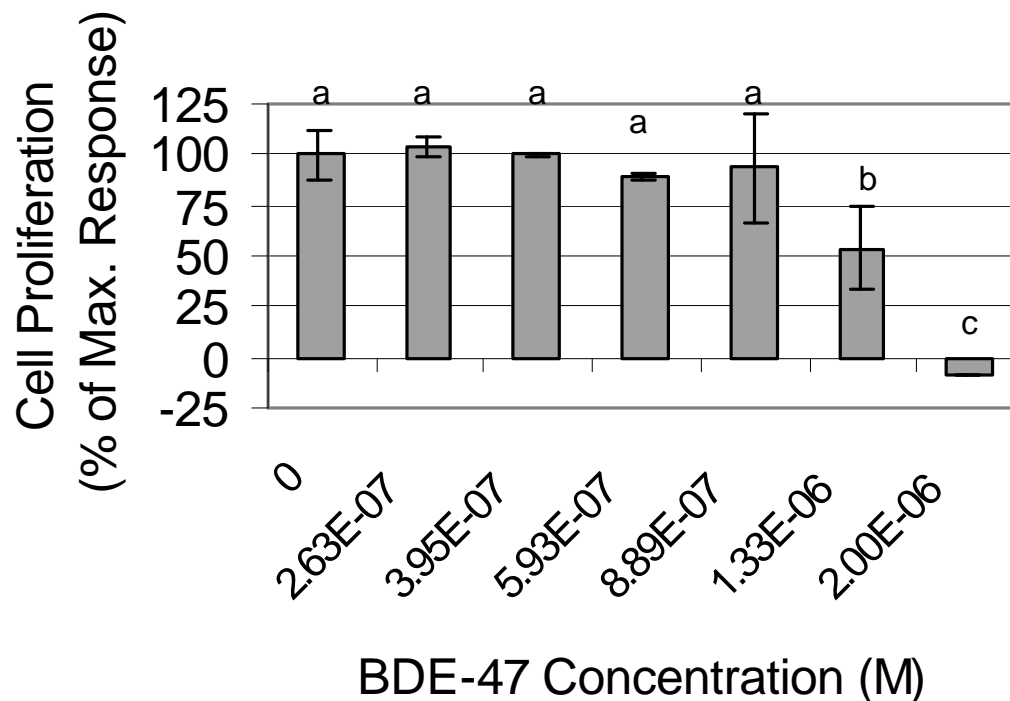


Figure 3. Proliferation of MCF-7 cells spiked with 100 pM  $17\beta$ -estradiol as a positive control and exposed to BDE-47 at the indicated concentrations. Error bars represent standard deviation ( $n=2$ ). Means with the same letter are not significantly different from each other (Student-Newman-Kuhls; SAS Version 8.2, SAS Institute 2001). Cytotoxicity is indicated where cell proliferation is significantly less than that of the control (or 100% of maximum response).

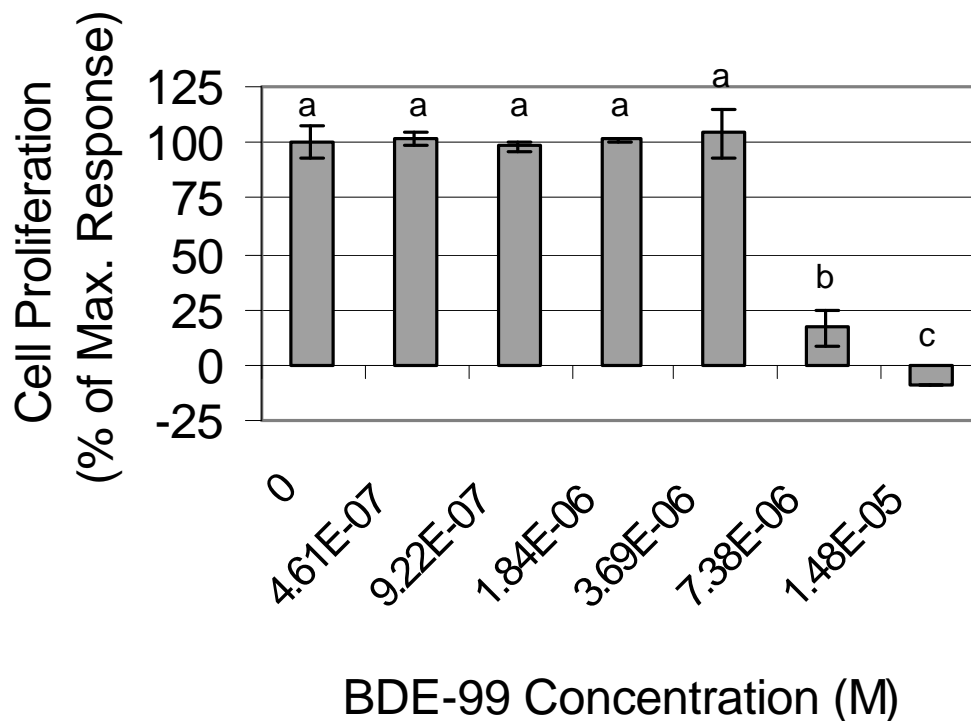


Figure 4. Proliferation of MCF-7 cells spiked with 100 pM 17 $\beta$ -estradiol as a positive control and exposed to BDE-99 at the indicated concentrations. Error bars represent standard deviation ( $n=2$ ). Means with the same letter are not significantly different from each other (Student-Newman-Kuhls; SAS Version 8.2, SAS Institute 2001). Cytotoxicity is indicated where cell proliferation is significantly less than that of the control (or 100% of maximum response).

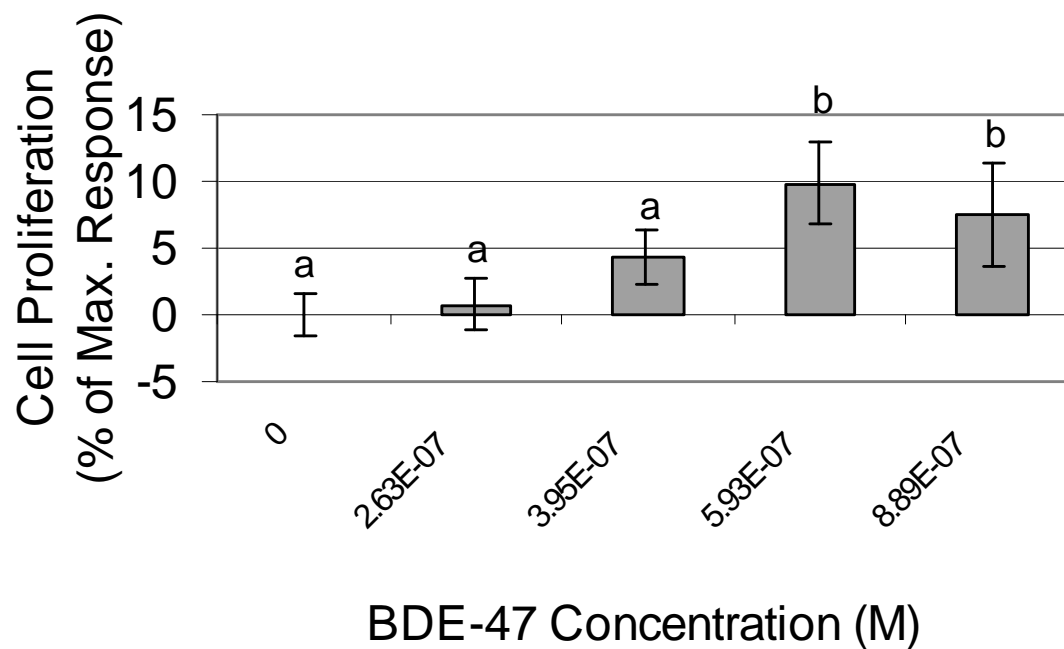


Figure 5. Proliferation of MCF-7 cells exposed to BDE-47 at the indicated concentrations. Error bars represent standard deviation ( $n=4$ ). Means with the same letter are not significantly different from each other (Student-Newman-Kuhls; SAS Version 8.2, SAS Institute 2001). Estrogenic activity is indicated where cell proliferation is significantly different from that of the control.

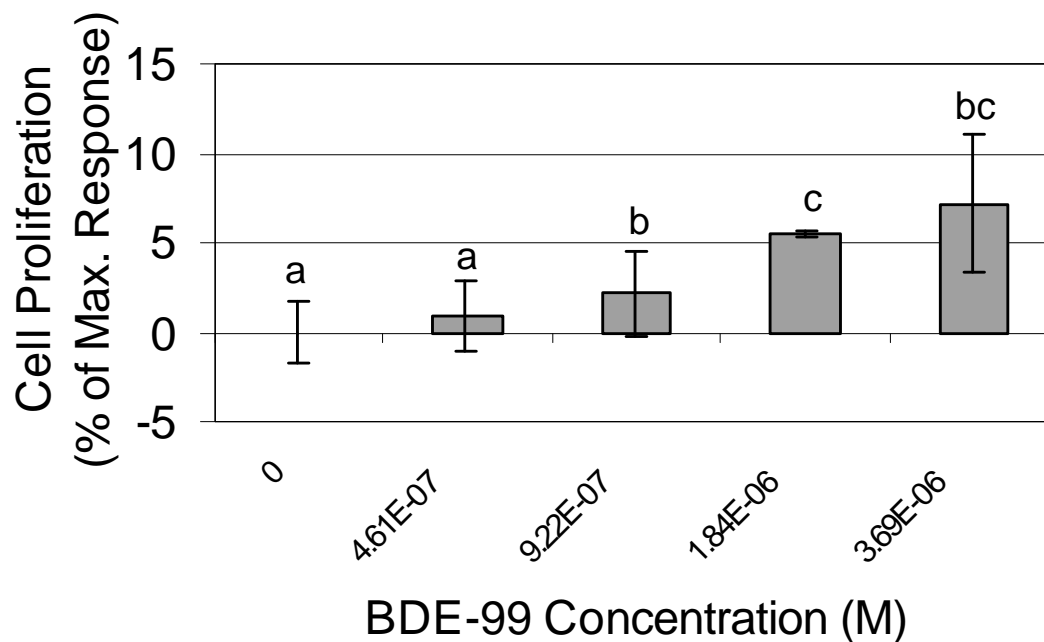


Figure 6. Proliferation of MCF-7 cells exposed to BDE-99 at the indicated concentrations. Error bars represent standard deviation ( $n=4$ ). Means with the same letter are not significantly different from each other (Student-Newman-Kuhls; SAS Version 8.2, SAS Institute 2001). Estrogenic activity is indicated where cell proliferation is significantly different from that of the control.

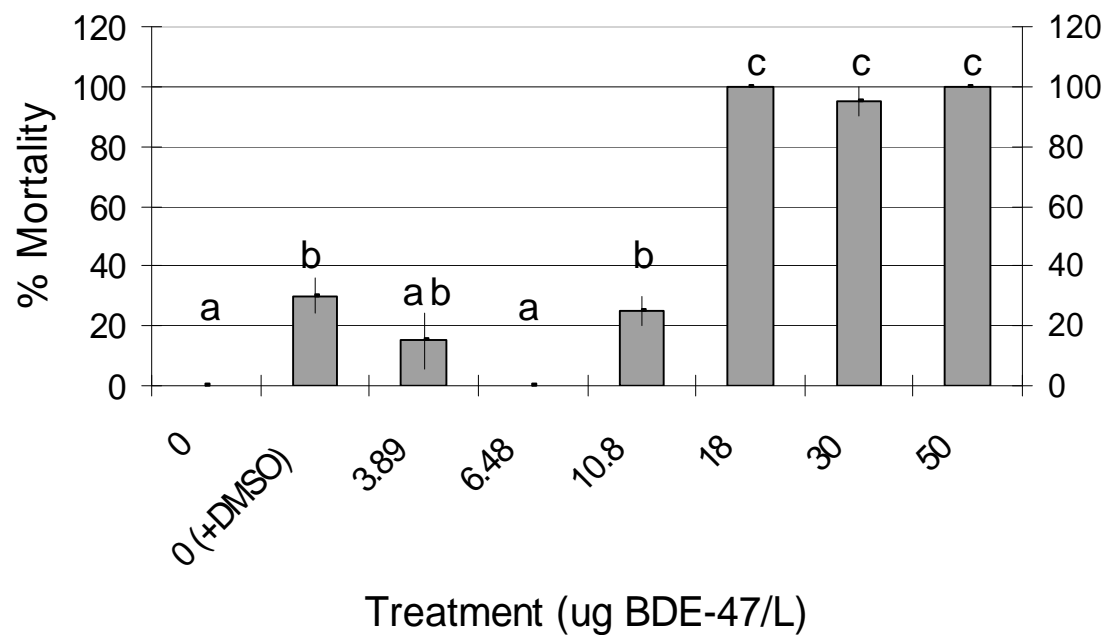


Figure 7. Mean ( $n=4$ ) % mortality ( $\pm$  standard error) of *C. dubia* exposed for 48 hours to eight different (nominal) concentrations of BDE-47 in the range-finding static renewal acute toxicity test. Means with the same letter are not significantly different from each other (Ryan-Einot-Gabriel-Welsch multiple range test; SAS Version 8.2, SAS Institute 2001).

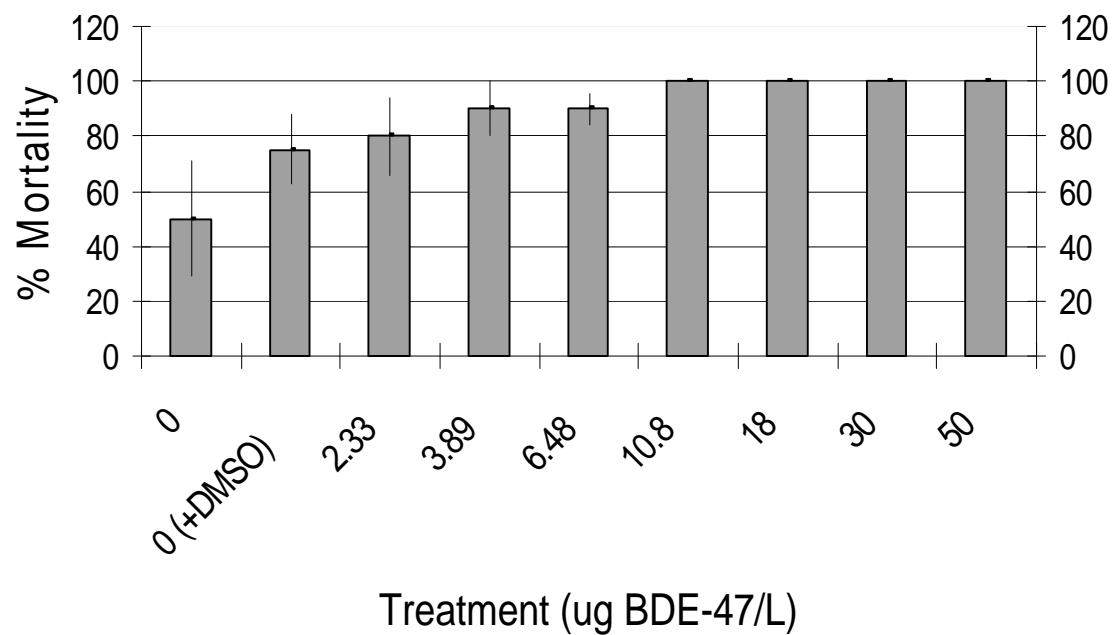


Figure 8. Mean ( $n=4$ ) % mortality ( $\pm$  standard error) of *C. dubia* exposed for 48 hours to nine different (nominal) concentrations of BDE-47 in the first static renewal acute toxicity test.

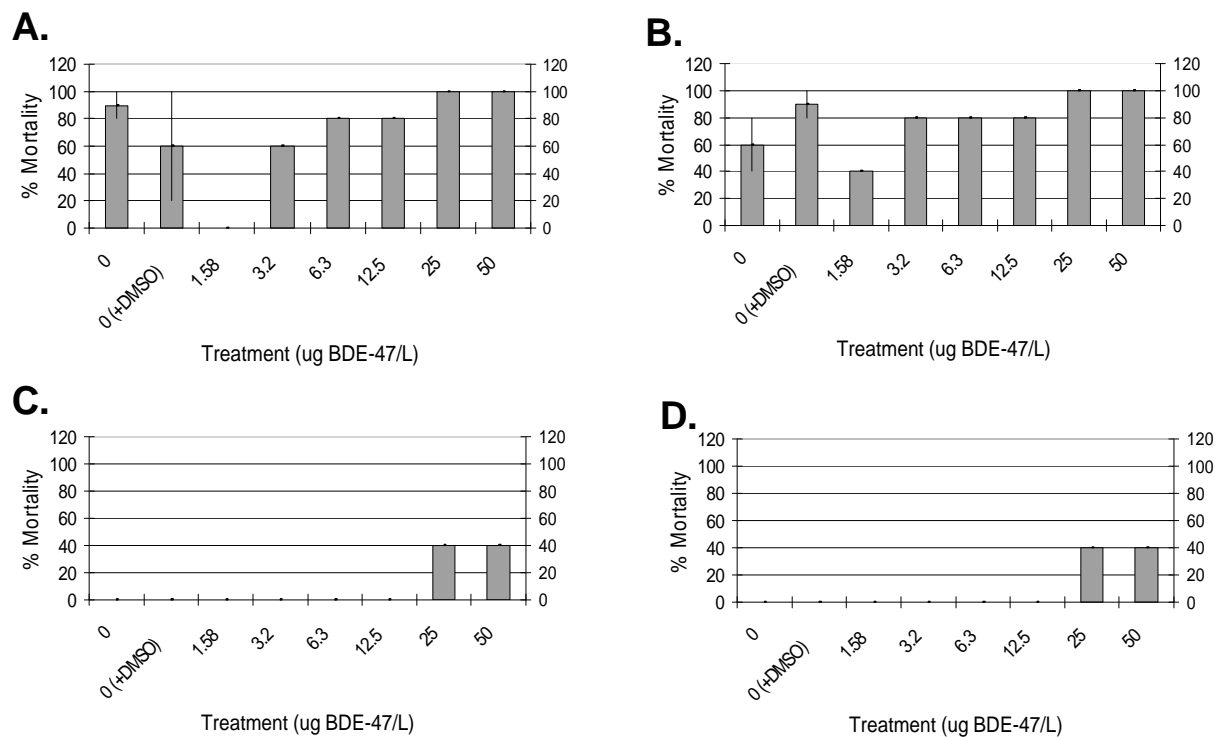
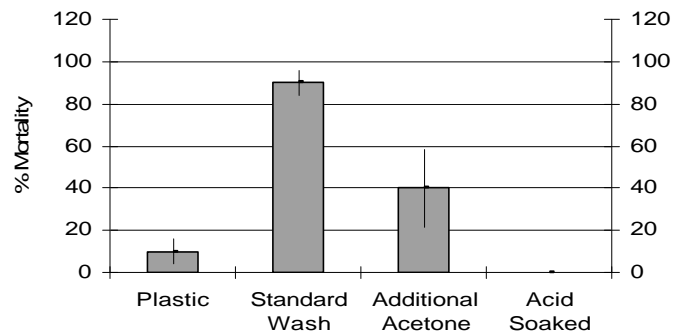


Figure 9. Mean ( $n=2$  for controls,  $n=1$  for BDE-47 treatments) % mortality ( $\pm$  standard error) of *C. dubia* exposed for 48 hours to eight different (nominal) concentrations of BDE-47 in each of four different beaker types: A) glass, covered; B) glass, uncovered; C) plastic, covered; D) plastic uncovered.

**A.**



**B.**

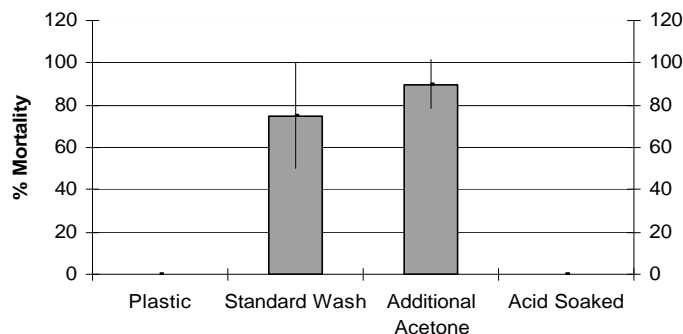


Figure 10. Mean ( $n=4$ ) % mortality ( $\pm$ standard error) of *C. dubia* exposed for 48 hours to A) dilution water or B) dilution water plus DMSO (0.5 mL/L) in beakers that had been washed one of four different ways: (1) not washed (disposable plastic beakers), (2) washed following standard operating procedure (SOP) for washing glassware (glass beakers), (3) washed following SOP with additional acetone rinse, or (4) acid soaked prior to being washed following SOP and acetone rinsed.

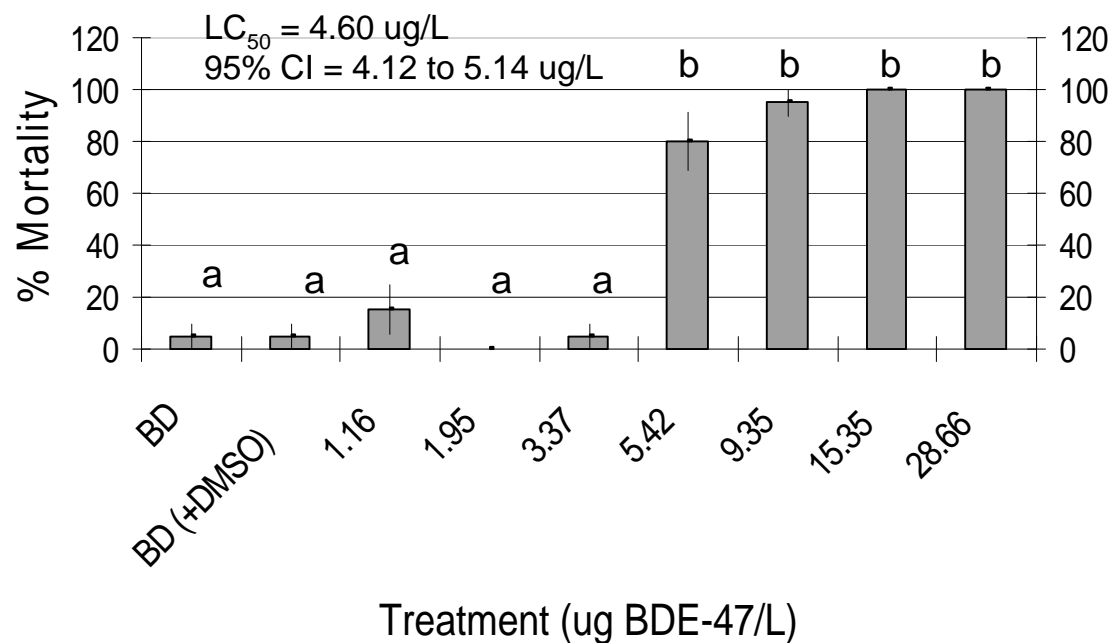


Figure 11. Mean ( $n=4$ ) % mortality ( $\pm$  standard error) of *C. dubia* exposed for 48 hours to nine different (measured) concentrations of BDE-47 in the second static renewal acute toxicity test. Means with the same letter are not significantly different from each other (Ryan-Einot-Gabriel-Welsch multiple range test; SAS Version 8.2, SAS Institute 2001). The lethal concentration for 50% of the population (LC<sub>50</sub>) value was calculated using the trimmed Spearman-Kärber method (Hamilton et al. 1977).

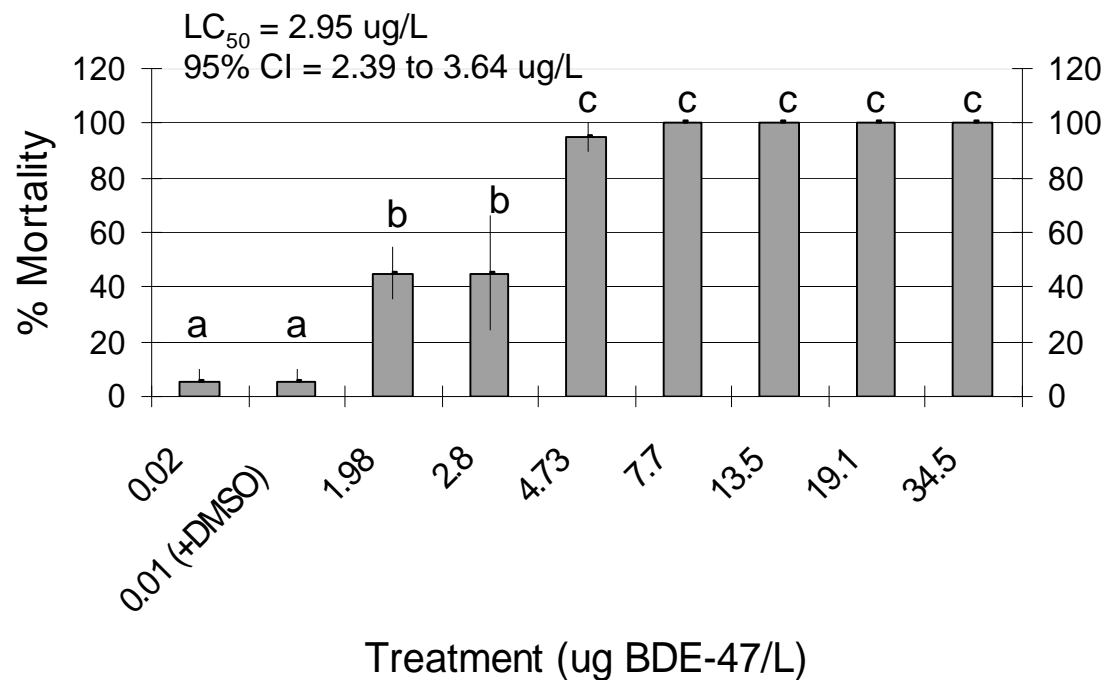


Figure 12. Mean ( $n=4$ ) % mortality ( $\pm$  standard error) of *C. dubia* exposed for 48 hours to nine different (measured) concentrations of BDE-47 in the third static renewal acute toxicity test. Means with the same letter are not significantly different from each other (Ryan-Einot-Gabriel-Welsch multiple range test; SAS Version 8.2, SAS Institute 2001). The lethal concentration for 50% of the population (LC<sub>50</sub>) value was calculated using the trimmed Spearman-Kärber method (Hamilton et al. 1977).

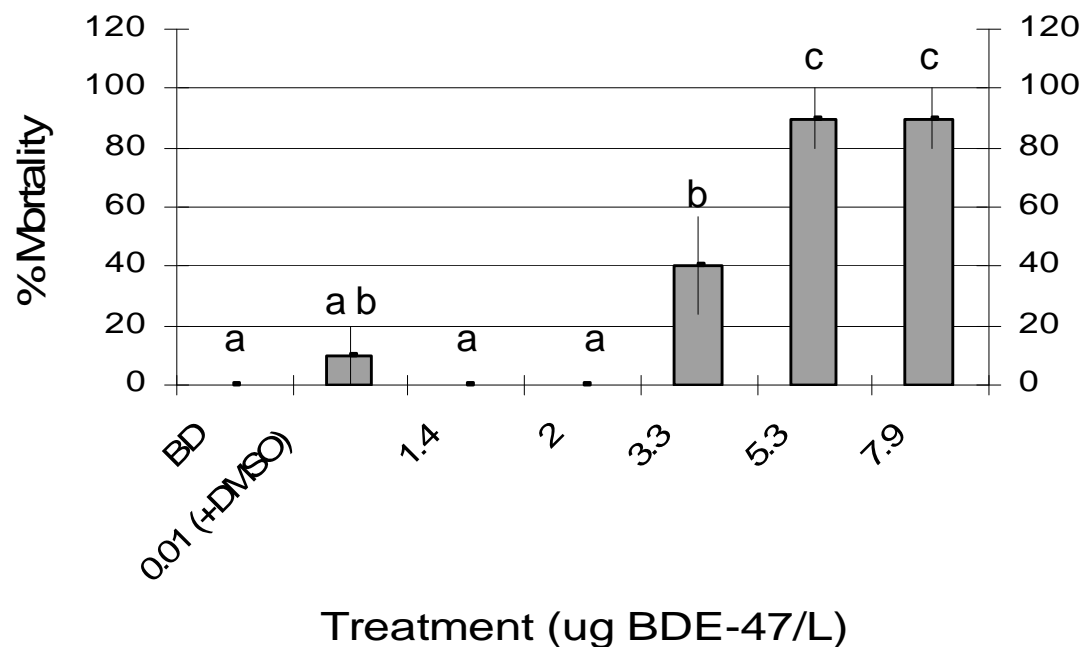


Figure 13. Mean ( $n=10$ ) mortality ( $\pm$  standard error) for *C. dubia* exposed for seven days to seven different (measured) concentrations of BDE-47 in the static renewal chronic toxicity test. Means with the same letter are not significantly different from each other (Ryan-Einot-Gabriel-Welsch multiple range test; SAS Version 8.2, SAS Institute 2001).

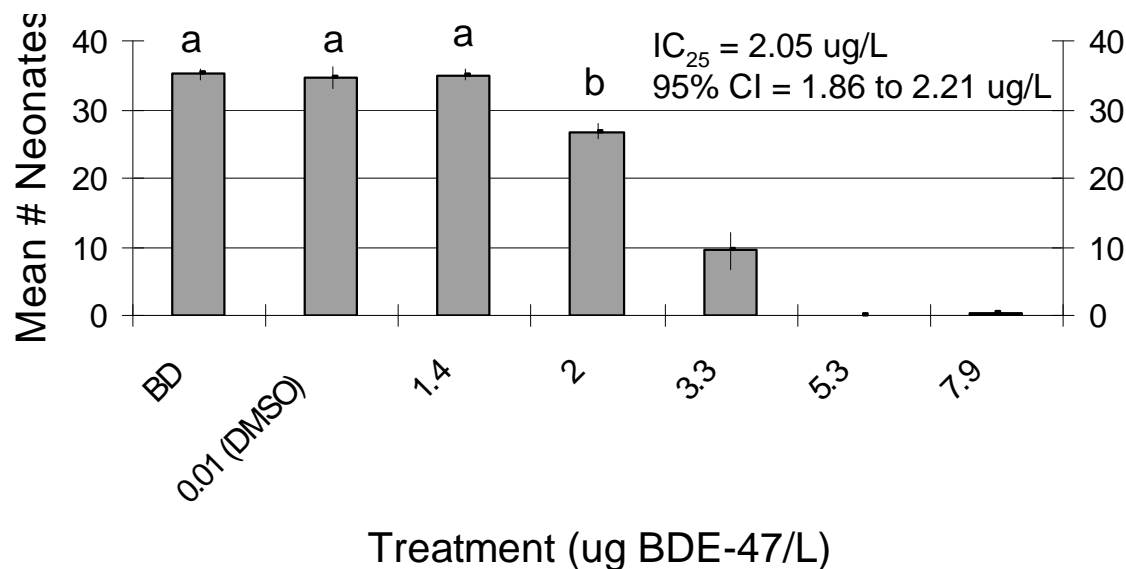


Figure 14. Mean ( $n=10$ ) number of neonates ( $\pm$  standard error) for *C. dubia* exposed for seven days to seven different (measured) concentrations of BDE-47 in the static renewal chronic toxicity test. Means with the same letter are not significantly different from each other (Ryan-Einot-Gabriel-Welsch multiple range test; SAS Version 8.2, SAS Institute 2001). Comparisons were conducted for those treatments with mortality not significantly different from the controls only. The inhibition concentration for 25% of the population (IC<sub>25</sub>) value (U.S. EPA 1993) is presented.